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Childhood obesity and multiple sclerosis: a Mendelian randomization study

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Abstract

Background: Higher childhood body mass index (BMI) has been associated with an increased risk of multiple sclerosis (MS).

Objective: To evaluate whether childhood BMI has a causal influence on MS, and whether this putative effect is independent from early adult obesity and pubertal timing.

Methods: We performed Mendelian randomization (MR) using summary genetic data on 14,802 MS cases and 26,703 controls. Large-scale genome-wide association studies provided estimates for BMI in childhood (n=47,541) and adulthood (n=322,154). In multivariable MR, we examined the direct effects of each timepoint and further adjusted for age at puberty. Findings were replicated using the UK Biobank (n=453,169).

Results: Higher genetically predicted childhood BMI was associated with increased odds of MS (OR=1.26/SD BMI increase, 95%CI 1.07-1.50). However, there was little evidence of a direct effect after adjusting for adult BMI (OR=1.03, 95%CI 0.70-1.53). Conversely, the effect of adult BMI persisted independent of childhood BMI (OR=1.43; 95%CI 1.01-2.03). The addition of age at puberty did not alter the findings. UK Biobank analyses showed consistent results. Sensitivity analyses provided no evidence of pleiotropy.

Conclusion: Genetic evidence supports an association between childhood obesity and MS susceptibility, mediated by persistence of obesity into early adulthood but independent of pubertal timing.

Introduction

Childhood obesity is a major public health problem, with more than a third of children in the United States being either overweight or obese, a trend which continues to increase.[1] Recently, observational studies have reported that obesity in early adulthood increases the risk of multiple sclerosis (MS).[2-6] While several studies also support a role for childhood obesity in MS,[4, 5, 7] some found that this association is no longer present following adjustment for obesity at a later life period.[2, 6] Understanding the role of early life risk factors is particularly important in MS, as the childhood period is a potentially critical window for environmental exposures, and potentially, for interventions.[8, 9] In addition, obesity is associated with a host of factors that can confound the association with the risk of acquiring the disease, which limits causal inference from traditional observational epidemiology.

Mendelian randomization (MR) is a strategy that can help address these limitations by harnessing natural genetic variation in populations to investigate the causal effect of a risk factor on an outcome.[10] This method greatly reduces confounding since allelic variants influencing different exposures are independently segregated and randomly assigned at conception.[11] The fact that genotypes are not modifiable by disease onset also limits reverse causation.[10] Previous MR studies have explored the relationship between obesity and MS but have reported on childhood or adult obesity separately.[12-15] Recent developments in MR can be applied to distinguish whether multiple exposures lie within the same causal pathway or if they have independent effects on the outcome.[16] Specifically, we have previously demonstrated the ability of genetic variants to separate between effects for childhood and adult body size using a multivariable MR framework.[17]

In this paper, we apply these methods to assess whether genetic predisposition towards higher childhood body mass index (BMI) increases the risk of MS. Then, we explore whether this effect is direct

or mediated by the persistence of obesity into early adulthood **(Figure 1)**. Similarly, we estimate the direct contribution of early adult obesity and further examine whether these associations are modified by pubertal timing. For this, we leveraged large-scale genome-wide association studies (GWAS) of MS, childhood and adult BMI (with a similar variance explained by the genetic instruments for both traits). We also provide independent replication using body size category estimates from the UK Biobank (UKB).

Methods

Genetic instruments for childhood and adult BMI

We identified genetic variants associated with childhood BMI using the largest GWAS meta-analysis of childhood BMI from the Early Growth Genetics (EGG) consortium, which included 47,541 children aged between 2 and 10 years (50.6% males).[18] Childhood BMI was calculated based on measured height and weight for more than 92% of participants. BMI values were transformed into standard deviation (SD) scores and adjusted for age and sex. Thus, the effect size for each allele represents a SD increase in childhood BMI, which in turn corresponds to a change in category from normal weight to overweight.[1, 19] To optimize power, we retained 23 single nucleotide polymorphisms (SNPs) that met either of the following criteria: (1) genome-wide significance (p-value < $5x10^{-8}$) in the joint analysis of the EGG consortium GWAS[18]; or (2) suggestive association (p-value < $5x10^{-6}$) with childhood BMI in EGG and genome-wide significance for the same variant with comparative body size at age 10 in the UKB (n=453,169) **(Dataset S1)**.[20] In a secondary analysis, we included only the 15 genome-wide significant SNPs satisfying the first criterion.

Effect estimates for adult BMI were obtained from a GWAS meta-analysis by the GIANT Consortium.[21] This included 322,154 individuals (47.1% males). Genetic associations were adjusted for age, age^2 and

sex, yielding 77 genome-wide significant variants in the European ancestry analysis **(Dataset S2)**. The average age of participants was 56 years,[21] which is beyond the usual age at MS diagnosis. However, previous studies have shown that a genetic risk score for BMI derived from those participants had a similar discriminative power in early adulthood (18-25 years) compared to middle adulthood, supporting the use of these variants to model early adult obesity in the context of MS (used interchangeably with adult obesity herein). For instance, the difference in BMI between the top and bottom fifth of genetic susceptibility in a Norwegian cohort was respectively 2.11 and 2.48 kg/m^2 for males and females aged 25, and 1.79 and 2.26 kg/m^2 for those aged 55.[22] Similar findings were reported in the Cardiovascular Risk in Young Finns Study, where the BMI difference between top and bottom genetic risk quartiles was approximately 2 kg/m^2 throughout early and middle-adulthood (18-49 years).[23] Importantly, these associations with BMI in early adulthood were replicated in other populations including U.S.[24, 25] and British[26] cohorts.

Genetic instruments for childhood and adult body size in UK Biobank

Genetic instruments for childhood and adult body size were obtained from our previous GWAS of 453,169 participants from the UKB.[20] Details have been described previously.[17] Briefly, our childhood measure of body size was based on recall questionnaire data asking individuals 'When you were 10 years old, compared to average would you describe yourself as thinner, plumper or about average?'. Adult measured BMI (age range 40-69 years) was converted into a categorical variable with 3 groups using the same proportions as the early life variable. GWAS were undertaken with adjustment for age, sex and genotyping chip using BOLT-LMM. Childhood body size analyses were additionally adjusted for month of birth to account for the individuals' relative age within their school year. This analysis yielded 298 and 557 independent genetic variants for childhood and adult body size respectively **(Datasets S3-4)**. We previously showed that this childhood body size measure displayed a strong genetic correlation

with childhood BMI estimates from the EGG consortium (r_g=0.85) and predicted childhood BMI in independent cohort (mean age 9.9 years).[17] Similarly, the categorical adult body size measure strongly correlates with the previous adult BMI effect from GIANT (r_g=0.96).

Genetic associations with MS

The effect estimates for each childhood and adult obesity-related variant on the risk of MS was obtained from the discovery cohorts of the latest GWAS meta-analysis by the International MS Genetics Consortium (IMSGC).[27] This dataset included 14,802 MS cases and 26,703 controls. When a genetic instrument for childhood or adult obesity measures was not present in another dataset, we identified a proxy SNP in linkage disequilibrium ($r^2 > 0.6$) using PLINK v1.9 and samples of European ancestry from 1000 Genomes project **(Datasets S7-9)**. All variants were aligned to the forward strand. In the case of childhood and adult BMI (not reported on GRCh37), forward strand alleles for palindromic SNPs were inferred for allele frequencies up to 0.42.

The presence of correlation between genetic instruments can lead to spurious MR estimates. Therefore, we ensured variant pairs were independent ($r^2 < 0.01$). We also excluded variants within the extended major histocompatibility complex (MHC) region given that its strong association with MS and complex linkage disequilibrium make it susceptible to horizontal pleiotropy (violation of MR assumptions). All genetic data were restricted to individuals of European descent to limit population stratification.

Univariable Mendelian randomization

We performed inverse-variance weighted MR to investigate the total effect of genetically increased childhood and adult obesity measures (i.e. BMI and body size) on MS susceptibility, using previously described methods.[28] A key MR assumption is that the genetic variants associated with the exposure

must not affect the outcome through pathways independent of the exposure (horizontal pleiotropy). We therefore conducted several sensitivity analyses to test this assumption.[29] First, we formally examined for overall horizontal pleiotropy using the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) Global test.[30] We also applied MR-PRESSO to exclude outlier and thus potentially pleiotropic variants. Second, we performed MR-Egger, a weighted linear regression allowing for the intercept to be estimated as a measure of average horizontal pleiotropy, as long as the pleiotropic effects are independent of the SNP-exposure effects.[31] Third, we performed a weighted median analysis, which provides robust estimates despite the presence of horizontal pleiotropy in a subset (<50%) of variants.[32] In addition, we inspected funnel plots of the individual MR estimates against their precision, with asymmetry indicative of directional horizontal pleiotropy.[31] Consistent results across these analyses reduce the likelihood of bias.

For the main analysis on childhood BMI, we also assessed whether genetic variants were associated with potential confounders by examining them in PhenoScanner,[33] a comprehensive database of genotype-phenotype associations, using a nominal p-value Bonferroni-corrected for the number of variants ($p < 0.05/23=0.00217$). Sensitivity analyses excluding variants associated with potential confounders were calculated.

To ensure that MR estimates did not suffer from weak instrument bias, we generated F-statistics for each genetic variant *j* using the formula:

$$F_j = \frac{\gamma_j^2}{\sigma_{Xj}^2}$$

where γ_j is the SNP-exposure estimate and σ_{Xj} is the corresponding standard error, and we reported the mean F-statistic for each obesity measure.

Last, to exclude the possibility that biological processes underlying MS influence childhood BMI, bidirectional MR was performed using non-HLA variants associated with risk of MS, with childhood BMI as the outcome **(Dataset S5)**.

Multivariable Mendelian randomization

We undertook multivariable MR to estimate the direct effects of childhood and adult obesity measures in turn on the risk of MS. Genetic instruments for each trait were included as in the univariable MR, after exclusion of outliers. For each variant, effect estimates on both childhood and adult obesity measures are included in a weighted multivariable regression model.[16] In addition, given the proposed role for pubertal timing in MS and its correlation with body weight status,[34, 35] we repeated the analysis with age at puberty as a third exposure (along with childhood and adult obesity measures) using genetic determinants of age at menarche as previously reported (n=329,245).[35, 36] The genetic architecture of pubertal timing across both sexes is highly correlated (r_g=0.75; p=1.2x10^{-79}), allowing these variants to provide insight into pubertal timing in males as well. Therefore, they are referred to as age at puberty variants.

All statistical analyses were performed in R (version 3.4.1). Statistical significance was set to $p<0.05$ unless otherwise specified. The data sources used in this study (IMSGC, EGG consortium, GIANT consortium, ReproGen consortium and UKB) obtained informed consent from all participants.

Results

Univariable Mendelian randomization

Univariable MR using the 23 childhood BMI-related SNPs showed that each 1-SD increase in childhood BMI was associated with 26% higher odds of MS (odds ratio [OR]=1.26, 95% confidence interval [CI] 1.07-1.50, p-value=0.006). A secondary analysis using the restricted set of 15 genome-wide significant SNPs yielded consistent results (OR=1.23, 95%CI 1.05-1.43, p-value=0.01). In PhenoScanner,[33] we identified associations with potential confounders between rs13387838 and smoking initiation (p=0.002), rs25832 and generalized epilepsies ($p=1.02x10^{-6}$), as well as between two variants and the number of years of schooling completed (rs13107325, p=0.002; rs7869969, $p=5.3x10^{-6}$). Exclusion of these four variants had no influence on the results (OR=1.29, 95%CI 1.12-1.49, p-value=0.0006). All observed SNP-phenotype associations are listed in **Dataset S10**; none were found for other reported MS risk factors including vitamin D levels, sun exposure, EBV or CMV infection, oral tobacco or organic solvent exposure. Similar findings were replicated using 277 childhood body size-related variants from the UKB (OR=1.40/change in body size category, 95%CI 1.10-1.78, p-value=0.01) **(Dataset S3)**.

These results were robust to sensitivity analyses for pleiotropy in both the EGG childhood BMI and UKB childhood body size analyses **(Figure 2)**. The MR-PRESSO global test showed no evidence of pleiotropy for the EGG childhood BMI analysis (p-value=0.093). In the larger UKB childhood body size analysis, the MR-PRESSO global test was significant ($p\text{-value}<2x10^{-5}$) yet the MR estimate after excluding 8 outlier variants **(Dataset S3)** remained largely unchanged (OR=1.38, 95%CI 1.09-1.76, p-value=0.008). The MR-Egger intercept was centered around zero (EGG childhood BMI: intercept=-0.020, 95%CI -0.052-0.012, p-value=0.22; UKB childhood body size: intercept=-0.001, 95%CI -0.008-0.006, p-value=0.72). The MR-Egger and weighted median estimates were consistent with the main finding **(Figure 2)**. Funnel plots were found to be approximately symmetric **(Figure 3)**. Using 188 non-MHC MS-related SNPs **(Dataset S5)**, we found no evidence that genetically increased MS susceptibility influenced childhood BMI (OR=1.01/log(odds) increase in MS, 95%CI 0.99-1.02, p-value=0.519).

Evaluating the effect of adult BMI on the risk of MS, univariate MR using 74 variants revealed a 40% increase in the odds of MS per 1-SD increase in BMI (OR=1.40, 95%CI 1.17-1.67, $p=3.1x10^{-4}$). The 522 genetic determinants of adult body size in the UKB identified a concordant association (OR=1.52/change in body size category, 95%CI 1.29-1.80, p-value=$8.2x10^{-7}$)

The mean F-statistics for obesity measures were all greater than 10, suggesting adequately strong instruments (childhood BMI: 45.2, adult BMI: 67.3, childhood body size: 66.9, adult body size: 52.0).

Multivariable Mendelian randomization

The direct effect of childhood obesity measures on the risk of MS was notably weaker compared to the total effect reported in the univariable analyses **(Figure 4)**. For childhood BMI, this direct effect (not mediated by adult BMI) was essentially null (OR=1.03, 95%CI 0.70-1.53, p-value=0.88). UKB childhood body size estimates similarly found evidence against a direct effect on MS (OR=1.05, 95%CI 0.64-1.73, p-value=0.84). The addition of age at puberty to the model did not alter the results (EGG childhood BMI: OR=1.10, 95%CI 0.68-1.78, p-value=0.70; UKB childhood body size: OR=1.02, 95%CI 0.59-1.74, p-value=0.95). In contrast, direct effect estimates for adult obesity measures remained robust and consistent with the total effects (**Figure 4**; GIANT adult BMI: OR=1.43; 95%CI 1.01-2.03, p-value=0.04; UKB adult body size: OR=1.75, 95%CI 1.35-2.27, p-value=$2.1x10^{-5}$). Inclusion of age at puberty did also not affect the estimates (adult BMI: OR=1.54, 95%CI 1.07-2.21, p-value=0.02; adult body size: OR=1.75, 95%CI 1,34-2.28, p-value=$3.4x10^{-5}$). The datasets used in the multivariable MR are presented in Datasets S6-9.

Discussion

This study examined the relative contribution of childhood and early adult obesity on the risk of MS using an MR framework. The results provide genetic support for a total causal effect of childhood obesity on MS susceptibility, albeit indirectly through persistence of larger body size in early adulthood. Indeed, when analyzed together with adult obesity measures, the contribution of childhood obesity was considerably attenuated and compatible with a null effect. In contrast, the association between adult obesity measures and MS remained unchanged after adjusting for childhood obesity measures, implying a direct effect on the risk of disease. Similar findings were obtained with both BMI measures and body size categories from independent cohorts, with the differences in effect sizes explained by the different scales (SD increase for BMI vs. change in body size category). We conducted several sensitivity analyses which found little evidence of genetic pleiotropy, adding further robustness to the findings. We have also shown that biological mechanisms underlying MS susceptibly do not have a large influence obesity measures. In addition, the inclusion of pubertal timing in multivariable MR did not influence the results, indicating that it does not lie within the causal pathway between obesity and MS risk. The finding corroborates our previous study showing that age at puberty does not exert independent effects on MS risk, but this does not eliminate the possibility that puberty attainment (rather its timing) may still play a key role in MS onset.[36]

These findings support previous observational studies that have examined the role of childhood obesity in MS. A prospective study reported a hazard ratio for MS of 1.15 to 1.18 per unit increase in BMI z-score measured between ages 7 and 13.[7] This was replicated in different populations using self-reported weight status.[4, 5] Those studies did not account for possible effects of obesity in later life. A study including women from two US cohorts reported a possible increased risk of MS with larger childhood body size at ages 5 and 10 using recalled silhouettes, however this association was no longer present

after adjusting for body size at age 20.[2] Conversely, larger body size at age 20 remained associated with MS despite adjustment for earlier time points. A case-control study similarly found that BMI at age 20, but not body size at age 10, was independently associated with MS risk.[6] Together with these observational findings, our results argue against a persistent effect of childhood obesity.

Previous MR studies have explored the relationship between obesity and MS but did not distinguish between childhood and adult time periods.[12-14, 37] Interestingly, our estimates for the total effect of adult BMI agree exactly with those from an earlier study using an independent MS susceptibility GWAS (OR=1.41, 95%CI 1.20-1.66).[13] Another MR study found no association between pediatric MS and an unweighted genetic risk score of 28 variants for childhood BMI,[37] although this could be attributable to the smaller sample size compared to the present study (569 vs. 14,802 cases) and the inclusion of weakly associated variants. More recent work reported a similar effect for childhood BMI on MS using the EGG consortium estimates, but did not account for adult obesity.[14]

The mechanisms by which obesity contributes to the etiology of MS are largely unknown. Proposed mechanisms include reduced circulating vitamin D levels and chronic inflammation secondary to increased adiposity.[15] In addition, higher BMI has also been shown to interact with environmental and genetic factors to increase risk of MS, namely EBV infection[38] and HLA-DRB1*15 status.[39]

An important strength of this study is the application of a two-sample MR approach which minimizes the risk of bias from confounding and allowed us to leverage large-scale genetic data on childhood and adult obesity measures as well as MS. Furthermore, we provide replication of our results using the UKB resource (n=453,169). We also build on recent methodological developments in multivariable MR to investigate time-dependent effects, an approach which may prove useful in other neurological

conditions. Nonetheless, we acknowledge a number of limitations. We cannot completely exclude the possibility of horizontal pleiotropy, which could bias the reported estimates. However, our sensitivity analyses included a range of methods that yielded generally consistent results. In selecting our genetic instruments for the EGG childhood BMI analysis, we included 8 variants that showed a suggestive association (p-value < $5x10^{-6}$) but were not genome-wide significant. This was justified by the gain in power and validated by their genome-wide significant association with body size at age 10 in the larger UKB. Repeating the analysis with only the 15 SNPs genome-wide significant in EGG resulted in similar point estimates across the various analyses. In addition, the inclusion of weak instruments in two-sample MR would be expected to drive the association towards the null. The imbalance in sample size between the EGG childhood BMI cohort (N=47,541) and GIANT adult BMI cohort (N=322,154) could have led to incomplete adjustment for childhood effects in multivariable MR. However, this possibility is lessened by the fact that both genetic instruments explained a similar variance in their respective traits (2.7% for childhood BMI and 2.3% for adult BMI). Moreover, the UKB analysis included the same number of individuals for both exposures. The adult BMI genetic estimates were derived from GWAS in middle-aged individuals older than the average age at MS onset. While subsequent studies support their influence over BMI in early adulthood, additional work is needed to better characterize age- and period-specific genetic effects using longitudinal measures and BMI trajectories throughout childhood, adolescence and adulthood. This study was restricted to individuals of European descent to prevent population stratification and therefore our findings have limited generalizability to other ancestral populations. European ancestry individuals were chosen because most GWAS data arises from individuals of this ancestry. This underlines the importance of on-going efforts to conduct GWAS in individuals of non-European ancestry. Last, the use of summary statistics precluded sex-stratified analyses as such genetic data sets are not currently available for MS. Reassuringly, previous cohort and

case-control studies have found no difference between MS risk and childhood or early adult obesity in males and females, although lower sample size for men generally led to reduced power.[6, 7]

In conclusion, this study provides genetic evidence supporting a role for childhood and early adult obesity in the development of MS. Importantly, our results also suggest that the effects of childhood obesity on MS are mediated by persistence of obesity into early adulthood, but independent of pubertal timing. These findings may help counsel individuals at high risk of MS and inform prevention strategies. Further investigation is required to better understand the biological mechanisms by which obesity influences the development of MS.

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Figure Legends

Figure 1. Directed acyclic graph of the possible pathways linking childhood obesity to MS susceptibility in a multivariable MR framework. Childhood obesity could influence the risk of MS directly (blue arrow), indirectly via persistence of obesity into early adulthood (orange arrow), or a combination thereof. In contrast, under a causal model, the total effect of early adult obesity should be equivalent to its direct effect (dotted line).

BMI = body mass index; MS = multiple sclerosis; SNP = single nucleotide polymorphism

Figure 2. Forest plot showing the univariable MR estimates investigating the total effect of childhood obesity measures on MS. There were no MR-PRESSO outlier variants identified in the EGG childhood BMI analysis. [a]Odds ratios for MS are reported per standard deviation increase in BMI. [b]Odds ratios for MS are reported per change in body size category.

BMI=body mass index; CI=confidence intervals; EGG=Early Growth Genetics; MR=Mendelian randomization; N_{SNPs}=number of variants in the analysis; OR=Odds ratio; SNP=single nucleotide polymorphism; UKB=UK Biobank.

Figure 3. Funnel plots of the individual MR estimates against their precision for the effect on MS of (A) childhood BMI and (B) childhood body size.

BMI=body mass index; EGG=Early Growth Genetics; IVW=inverse-variance weighted; MR=Mendelian randomization; SE=standard error; UKB=UK Biobank.

Figure 4. Forest plot illustrating the total and direct effects of childhood and adult obesity measures on MS. Effects from univariable MR are post outlier removal when applicable. [a]Odds ratios for MS are reported per standard deviation increase in BMI. [b]Odds ratios for MS are reported per change in body size category.

BMI=body mass index; CI=confidence intervals; EGG=Early Growth Genetics; MR=Mendelian randomization; N_{SNPs}=number of variants in the analysis; OR=Odds ratio; SNP=single nucleotide polymorphism; UKB=UK Biobank.